1. **RT-PCR**

2. **RT with random primers**
    a. **Mix and briefly centrifuge each component before use**
    b. **Add 2ul (1-5ul) sample RNA or the same volume of DEPC H2O to a sterile tube.**
    c. **Prepare a master mixture in sterile 0.5ml tubes as follows**
    d.

Total sample
1ul 10 mM dNTPs
1ul Random primer
6ul DEPC treated H2O

    e. **Add 8 ul the master mixture to each sample tube, incubate at 65^0C for 5 minutes, and then place on ice for at least 1 min.**
    f. **Prepare the following reaction mixture (adding each component in the indicated order)**

        2ul 10x RT buffer
        4ul 25 mM MgCl2
        2ul 0.1 M DTT
        1ul Rnasin (or similar RNAse inhibitor)

        Add 9ul of reaction mixture to each RNA/primer mixture, mix gently, and briefly centrifuge.

    g. **Incubate at 25^0C for 2 min.**
    h. **Add 1ul of SUPERSCRIPT II RT to each tube, mix and incubate at 25^0C for 10 min.**
    i. **Transfer the tubes to 42^0C and incubate for 50 min.**
    j. **Terminate the reaction at 70^0C for 15 min and chill on ice.**
    k. **Briefly centrifuge the sample down to the bottom and add 1ul of Rnase H to each tube and incubate for 20 min at 37^0C.**
    l. **Add 30ul DEPC H2O, and store at - 20^0C before PCR. (optional based on expression level of product)**

3. **PCR**

    a. **Prepare the following reaction mixture**

        5ul cDNA
        5ul 10xPCR buffer 15mM MgCl2 (Perkin Elmer)

        10ul 1 mM dNTPs

1 ul 0.1ug/ul sense primer
1 ul 0.1ug/ul anti-sense
27.5 ul distilled water

b. **Add 44.5ul PCR mix to each tube.**
c. **Add 5ul cDNA samples and a drop of mineral oil.**
d. **Put the sample tubes into PCR machine.**
e. **Start file # 62 for a hot start at 95^0C for 10 minutes.**
f. **Stop file #62, add 0.5ul of AmpliTaq Gold polymerase (Perkin Elmer).**
g. **Enter file #75 and start.**